REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	02/28/97	Performance Report 3/1/96-2/28/97

4. TITLE AND SUBTITLE
"Genetic Regulation in the Aiptasia pallida Symbiosis" - Performance Report Year 1

5. FUNDING NUMBERS

6. AUTHOR(S)
Dr. Jean-Francois Tomb

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Institute for Genomic Research
9712 Medical Center Drive
Rockville, MD 20850

8. PERFORMING ORGANIZATION REPORT NUMBER
ONRYR1

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
Ballston Centre Tower One
800 North Quincy Street
Arlington, VA 22217-5660

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release

12b. DISTRIBUTION CODE

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

13. ABSTRACT *(Maximum 200 words)*

This report describes the progress of the project "Genetic Regulation in A. pallida Symbiosis". The main goal of the project in year 1 was to identify sequence tags for differentially expressed genes using the SAGE approach. The initial protocal we followed to compare gene expression in cultured and symbiotic zooxanthellae is one developed for serial analysis of gene expression (SAGE). We initially tested the SAGE protocol with cDNA generated from K. rotundatum. Unfortunately, to date little progress has been made towards meeting our initial goal. The application of the SAGE technique proved technically difficult. We are now focusing on constructing representative cDNA libraries from cultured and symbiotic zooxanthellae and will sequence from both the 3'- and 5'-end approximately 5,000 clones from each library and compare the results. This work will be conducted throughout years 2 and 3.

14. SUBJECT TERMS	15. NUMBER OF PAGES
-sequence tags -EST approach -serial analysis of gene expression (SAGE)	2
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
unclassified	unclassified	unclassified	unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Progress Report
Office of Naval Research

"Genetic Regulation in A. pallida Symbiosis"
Grant #: N00014-96-1-0604

This report describes the progress of the project "Genetic Regulation in A. pallida Symbiosis". The main goal of the project in year 1 was to identify sequence tags for differentially expressed genes using the SAGE approach.

Since receiving the award from ONR, we concentrated our initial efforts on the zooxanthellae. Isolation of mRNA and cDNA synthesis procedures were selected and tested with yeast and then with a different (non-zooxanthellate) cultured dinoflagellate, *Katodinium rotundatum*. These tests led to an effective protocol. We received cultured zooxanthellae from Dr. Clay Cook, Harbor Branch Oceanographic Institute and proceeded to isolate mRNA and construct cDNA from these samples.

The initial protocol we followed to compare gene expression in cultured and symbiotic zooxanthellae is the one developed for serial analysis of gene expression (SAGE) by Velculescu *et al* (1995)[1]. When successful, SAGE results in the generation of many short (9-12 base pair) tags concentrated in a single sequence. The plan was then to compare frequency diagrams of the tags derived from the expressed genes of the two groups of zooxanthellae to determine which tags represent differentially expressed genes. Tags from these genes were then to be used as probes against full cDNA (expression) libraries and selected clones were to be sequenced.

We initially tested the SAGE protocol with cDNA generated from *K. rotundatum*. Unfortunately, to date little progress has been made towards meeting our initial goal. The application of the SAGE technique proved technically difficult despite repeated efforts by a skilled technician. We are now focusing on constructing representative cDNA libraries from cultured and symbiotic zooxanthellae and will sequence from both the 3'- and 5'-end approximately 5,000 clones from each library and compare the results. This work will be conducted throughout years 2 and 3. This EST approach, though not exhaustive, will identify a large number of zooxanthellae genes and will establish a reference database for future work. In addition, it will allow the identification of differentially expressed genes.

---

[1]V.E. Velculescu, L. Zhang, B. Vogelstein, K.W. Kinzler. 1995. Serial Analysis of Gene Expression. Science, 270: 484-487.

The budget for year 1 has not been spent in large part due to the problems with progress on the project. As of February 28, 1997, $56,930.75 total costs was carried over to year 2. The majority of the carryover is related to materials and supplies. We expect to spend all of the carryover funds from year 1 and all of year 2 funds by the end of the second year.